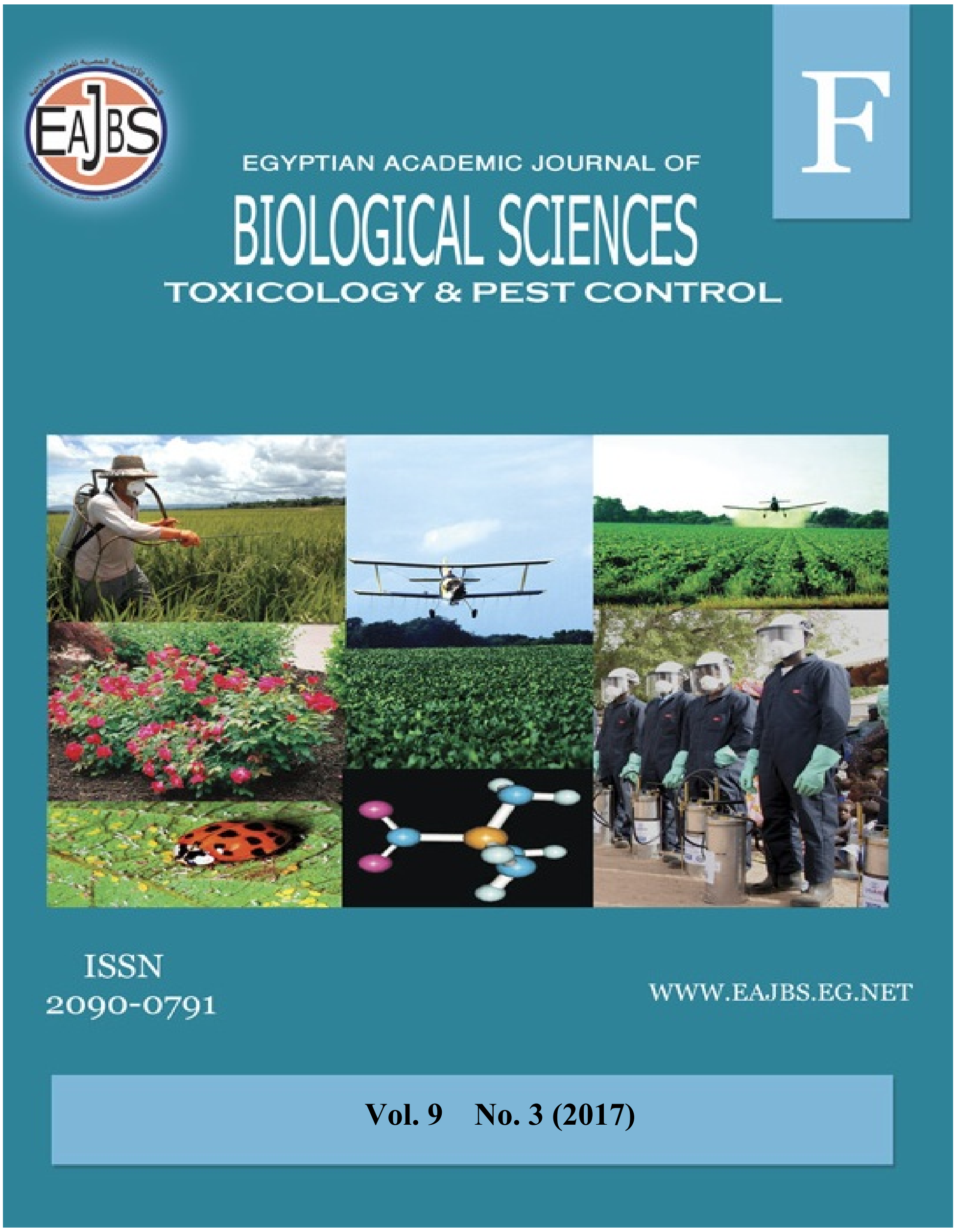

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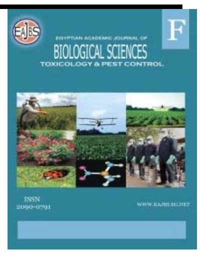

Relationship Between *Bemisia tabaci* and *Aphis gosspyii* Infestations with Certain of Plant Diseases, Plant Enzyme Activities, Dissection Structures and Natural Enemies on Squash Plant.

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ABSTRACT

The whitefly *Bemisia tabaci* and cotton aphid, *Aphis gosspyii* are considered as the most harmful insect pests to squash, *Cucurbita pepo* L. crop. Beside the direct damages, white fly transmits pathogenic virus and induces plant physiological disorders, as the silver leaf disorder and cotton aphid transmits mosaic virus. In this study, we evaluated the development of leaf silvering and mosaic virus in squash plant exposed to the infestation of *B. tabaci* and *A. gosspyii*, respectively. An experiment was conducted under field conditions, in Mansheyet Saqqara village, Giza, Egypt, during the summer seasons, 2016 and 2017.

The activity of some plant enzymes, such as Alpha Esterase, Peroxidase & Phenoloxidase and chlorophyll contents were correlated with *B. tabaci* and *A. gosspyii* infestations. The associated predators, mirid bug, *Nesidiocoris tenuis* and ladybird, *Coccinella undecimpunctata* were collected and their relation was also studied. The leaf silvering degrees were also determined during this study.

The results indicated that induced silver leaf in squash cultivar was similar to a systemic phytotoxemia, mild symptoms in which silvering along the main and secondary veins. While, sever symptoms included completely silvering were observed on the upper leaf surface. Inversely, the lower leaf surface appeared normal.

INTRODUCTION

The whitefly, *Bemisia tabaci* and cotton aphid, *Aphis gosspyii* are caused severe damages to squash crop, *Cucurbita pepo* L.. They cause direct damage by continuous sucking of plant xylem (Bleicher *et al*., 2000) and silver leaf disease (Lourenção *et al*., 2011), sooty mold development and mosaic virus disease as an indirect damage (Nagata *et al*., 2005), that reduce plant productivity. The silvering leaf appearance due to separation of the upper epidermis from the palisade mesophyll resulting in altered reflection of light (Jimenez *et al*., 1995 and Burger *et al*., 1988). This appearance of silvering suggests that some trans-located factor moves from the infested leaves via the phloem to the apical meristem and the newly developed leaves (Yokomi *et al*., 1990). In addition, chlorophyll levels were lowered by as much as 40% to 50% as a result of whitefly feeding (Jimenez *et al*., 1995 & Yokomi *et al*., 1995). These increased light reflectance and reduced chlorophyll which will cause a reduction in photosynthesis (Jimenez *et al*., 1995).

Aphids attack of vegetables, grains, tree crops, flowers and ornamentals plants. High population of aphids reduced yield and crop quality due feeding damage, causing significant problems due to honeydew and the transmission of numerous viruses of plants (Oerke and Dehne, 1997).

Among the most important aphid species, *A. gosspyii* was considered as a major pest attacking the Cucurbitaceae plants (Quiros *et al*., 2006). The cotton aphid, *A. gosspyii* caused mosaic disease which resulted in severe symptoms on squash leaves and fruit (Provvidenti *et al*., 1984) and was caused by mosaic virus (Lisa *et al*., 1981). This disease was characterized by severe mosaic and distortion of younger leaves and diagnostic bumps and distortion of the fruit. This virus' vector is an aphid, and its incidence should not be related directly to whiteflies.

The purpose of this investigation has been to evaluate the influence of *B. tabaci* and *A. gosspyii* populations on squash plant and their relation to silver leaf degrees, mosaic virus percent, some dissection structures and certain natural enemies. Besides, the effect of both pests on the activity of some plant enzymes in squash plant.

MATERIALS AND METHODS

In order to study the development of leaf silvering and mosaic virus in squash plant, *Cucurbita pepo* L. submitted to the infestation of *Bemisia tabaci* and *Aphis gosspyii*, respectively. An experiment was conducted under field conditions, in Mansheyet Saqqara village, Giza, Egypt, during the summer seasons, 2016 and 2017. An area of 525 m^2 was divided into three plots and was sown by seeds of squash plant, *Cucurbita pepo* L. at 15th March 2016 and 2017. In order to estimate the infestation of *B. tabaci* nymphs and adults along with *A. gosspyii* individuals, samples of 30 leaves from the three replicates were randomly picked at weekly intervals started from the date of sowing (15th March, 2016 and 2017) until the end of this experiment. Each sample was kept in a tightly closed paper bag and transferred to the laboratory in the same day for inspection. Numbers of *B. tabaci* nymphs and adults stages and *A. gosspyii* individuals were counted and recorded.

The leaf-silvering evolution was evaluated in squash cultivar through a graded scale varying from 0 to 5, adapted from the scale proposed by Paris *et al*. (1987). The grade 0 means absence of symptoms on leaves and the others indicate the gradual development of the symptoms, it means, degree 1, silvering in and parallel to less than one-half of the leaf veins; degree 2, silvering in and parallel to more than half, but not all, of the leaf veins; degree 3, silvering in and parallel to all leaf veins; degree 4, all veins and some spaces between veins silvered; degree 5, entire upper leaf surface silvered (Fig. 1).

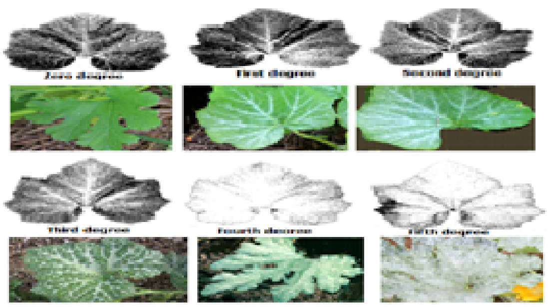


Fig. 1: Grading scale for squash silver leaf, ranging from Degree 0, Degree 1, Degree 2, Degree 3, Degree 4 and Degree 5 (According to Paris *et al*., 1987).

These silvering degrees were calculated per weekly sample of 10 plants for each replicate. The mosaic virus percent was also calculated for weekly sample of 50 plants. The associated predators, mirid bug, *Nesidiocoris tenuis* and ladybird, *Coccinella undecimpunctata* were collected and their relation with the two pests was also studied.

To study the relationship between the activity of some plant enzymes, such as Alpha Esterase (Ni *et al.*, 2001 and Van Asperen, 1962), Peroxidase (Vetter *et al.*, 1958) and Phenoloxidase (Ishaaya, 1971) and chlorophyll contents (Witham *et al*., 1971) and *B. tabaci* and *A. gosspyii* populations, leaf silvering degrees and mosaic virus percent were determined at Chemical Analysis Constituent, Insect Physiology Dept., Plant Protection Research Institute, Agricultural Research Institute (ARC). The slides of leaf layers were made at plant Dept., Faculty of science, Ain shams University, and then, related with the infestations. Samples of squash leaves were taken at seedling, flowering and fruiting) stages and placed in a fixed solution FAA (15cm Formalin + 85cm ethyl alcohol + 5cm glacial acetic acid) four 48 hours then transferred into 70% ethanol alcohol. Then after, a transverse sections in there leaves were made by using the method described by Jackson (1976). The different measurements (in micron) of the thickness of upper epidermis layer, palisade tissue, spongy tissue and lower epidermis layer were determined by using of Compu Eye, Leaf and Symptom Area Program by Bakr (2005).

Statistic procedures: The data were submitted to analysis of variance and the means were compared using t-test, F-test, simple correlation and partial regression using SAS program computer version 9.3 (SAS, 2003).

RESULTS AND DISCUSSION

Pests:

Cotton whitefly, *Bemisia tabaci* and silvering leaf symptoms:

The first investigation was performed after two weeks from the cultivation, there had an average scores of 0.73 and 0.54 nymphs/ leaf and 0.00-0.18 adults/ leaf during summer seasons, 2016 and 2017, respectively (Table 1). Also , there scored two peaks of nymphs and adults of *B. tabaci* during both two successive season being 2.96 and 5.23 nymphs per leaf at April, 15th and May, 6th, respect., during season, 2016, and 3.97 and 7.42 nymphs/ leaf at summer season, 2017.

Table 1: Incidence of *B. tabaci* and *A. gosspyii* individuals and their damage symptoms on squash plant during two summer seasons, 2016 and 2017.

Inspection dates	Mean no. of *Bemisia tabaci*				No. of silvering leaves/ 10 plants												Mean no. of *Aphis gosspyii* individuals/Leaf		No. of virus/ 50 plants		Mosaic virus %	
					2016						2017											
	Nymphs/Leaf		adults/Leaf		Degree of silvering														2016	2017	2016	2017
	2016	2017	2016	2017	1st	2nd	3rd	4th	5th	Total	1st	2nd	3rd	4th	5th	Total	2016	2017				
April, 1st	0.73	0.54	0.00	0.18	6	0	0	0	0	6	10	2	2	2	2	18	0.54	0.33	0	2	0	4
8th	1.6	0.84	0.39	0.41	2	2	1	1	2	8	5	5	4	4	5	23	7.71	21.9	4	7	8	14
15th	2.96	3.97	2.36	1.29	5	2	3	9	7	26	6	3	4	10	8	31	27.49	9.69	8	9	16	18
22nd	2.1	1.54	1.25	0.86	0	7	6	4	2	19	1	8	7	5	3	24	5.28	15.64	2	3	4	6
29th	1.56	7.42	2.43	0.74	4	2	0	0	5	11	9	7	5	5	10	36	5.81	25.49	8	13	16	26
May,6th	5.23	5.5	2.61	1.29	3	7	9	4	2	25	5	9	11	6	4	35	6.47	6.82	3	5	6	10
13th	2.5	5.27	1.53	1.07	5	2	3	9	7	26	4	4	7	14	4	33	0.73	8.35	8	6	16	12
20th	2.04	1.48	0.37	0.20	2	2	5	12	2	23	7	4	5	11	9	36	0.63	0.18	4	10	8	20
27th	1.84	0.5	0.27	0.10	5	10	4	4	2	25	7	12	6	6	4	35	0.34	0.06	2	4	4	8
June, 3rd	0.00	0.00	0.00	0.00	3	7	8	15	2	35	5	9	10	17	4	45	0.00	0.00	3	5	6	10
Overall mean	**2.06**	**2.71**	**1.12**	**0.61**	**20.40**						**31.60**						**5.50**	**8.85**	**4.20**	**6.40**	**8.40**	**12.80**
Prob.	0.2430 NS		0.0208 *		0.0094 *												0.1455 NS		0.1351 NS		——	

However, the squash, *C. pepo* plants showed no observations of *B. tabaci* adults at the first inspection during season, 2016, whereas, a few average numbers of *B. tabaci* adults (0.18) was scored at the same last inspection. Similarly as soon as in case of nymph, the *B. tabaci* adults infested squash plants by two peaks being 2.36 and 2.61 and 1.29 &1.29 adults/ leaf at April, 15th and May, 6th during two successive seasons, 2016 and 2017, respectively. The overall infestations of *B. tabaci* nymphs and adults was 2.06 – 2.71 nymphs/ leaf and 1.12 – 0.61 adults/ leaf during 2016 and 2017 seasons, respectively. There were significant differences in case of *B. tabaci* adults but an insignificant difference in case of nymphs (Table 1).

Silvering of foliage is an important disorder disease in summer squash in Egypt, silvering leaf disorder disease included silvering of the upper leaf surface whereas, the lower surface of squash leaf was appeared normal. The silvering degrees forms aggradation from full green (no-silvering) to completely entire silvering on the upper squash leaf during the summer seasons, 2016 and 2017 (Fig. 1). Silvering symptoms was expressed in and parallel to leaf veins as illustrated in Fig. (1), except in severe cases as 5th degree when it completely encompassed the entire squash leaf (Burger *et al.*, 1983). The silvering of 1st degree appeared at the first inspection being 6 silvering leaves/ 10 plants of the sample during summer season 2016, whereas, all of silvering degrees from 1st to 5th during season, 2017 (Table 1). In the present experiment, silvering disorder of 1st was ranged between 0-6 and 1-10 of silvering leaves per 10 plants during in both two seasons, respectively. The silvering squash leaf was developed from 0-10, 0-9, 0-15 and 0-7 for 2nd, 3rd, 4th and 5th degrees during summer season, 2016, respectively (Table, 1). However, it was ranged from 2-12, 2-11, 2-17 and 2-10 for 2nd, 3rd, 4th and 5th degrees during summer season, 2017, respectively. Generally, significantly silvering occurred during two summer seasons, 2016 and 2017 being 20.40 and 31.60 of silvering leaves /10 plants (probability= 0.0094). Once silvering appeared in squash leaf, it remains in that leaf as permanent symptom; the newly developed leaves were appeared green until infested by *B. tabaci* stages. In the current investigated, the 4th, 5th degrees were heavy silvering, and were reflected more light than other silvering degrees (Shifriss, 1983). Like pubescence, silvering severs to increase reflectance, and then silvering can be developed the desiccation of squash plants (Paris *et al.*, 1987). A little infestation of *B. tabaci* individuals caused physiological disorders (silvering squash leaf SSL) which reducing transpiration and photosynthesis. Similarly, numerous studies also demonstrated that the association between the feeding of *B. tabaci* individuals and the squash leaf silvering disease (Paris *et al.*, 1987 and Lourenção *et al.*, 2011).

Cotton aphid, *A. gosspyii* and mosaic virus:

A. gosspyii pest was slightly observed in the 1st week of infestation, but it was rapidly increased till reach the maximum (being 27.49 aphids/ leaf) at April, 15th 2016. Then, it rapidly decreased until disappeared in the end of summer season, 2016 (Table, 1). During summer season, 2017, aphid populations gradually increased until reach to their maximum numbers (25.49 aphids/ leaf) during last week of April, 2017 (Table, 1). Results were confirmed by those results obtained by Benerjee *et al*. (1986) and El-Khawas and El-Khawas (2008). The populations of *A. gosspyii* individuals were arranged between 0 - 27.49 and 0 - 25.49 aphids/ leaf with overall mean numbers being 5.50 and

8.85 aphids. There were nonsignificant differences between them (Tables 1). Feeding of *A. gosspyii* individuals on leaves of squash plantations cause leaf crumple and mosaic virus which can further result in yield loss. Mosaic virus is poorly aphid transmissible at first observation at April, 1st during two successive seasons, 2016 and 2017. The number of mosaic virus expended between 0-8 and 2-13 of virus/ 50 plants during two seasons, 2016 and 2017, respectively, there were nonsignificant difference between them (Table 1). Mosaic virus disease percent was recorded the highest percent at 15th April 2016 and the last week of April, 2017, respectively (Table 1). While, mosaic virus progression was similar during seasons, 2016 and 2017, suggested that *A. gosspyii* infestation was a slight infestation (Table 1). The overall mosaic virus percent which transmitted by aphid was 8.40% and 12.80% during seasons, 2016 and 2017, respectively. Promising results recently obtained by Wang *et al.* (1991) in which a variant mosaic virus was transmitted to summer squash plantations by *A. gosspyii*. The variant mosaic virus caused mild symptoms both cucurbits, especially squash plantations (El-Kady, 2007). In general, the reduced transmission rate of mosaic virus to squash plantations might be due to reduce the aphid infestation during summer plantations. It can be concluded that the summer squash plantations need to demonstrate the Integrated Pest Management (IPM) tactics for aphid control to avoid the transmission of mosaic virus to squash plantations.

Interaction between associated predators and damage symptoms with *B. tabaci* and *A. gosspyii* infestations:

The most common observed predators found associated with the current piercing sucking insect pests were zoophytophagous mirid bug, *Nesidiocoris tenuis* (Heteroptera: Miridae) and *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae). The population incidence of *N. tenuis* was studied under field conditions at weekly intervals during summer seasons, 2016 and 2017 (Fig. 2).

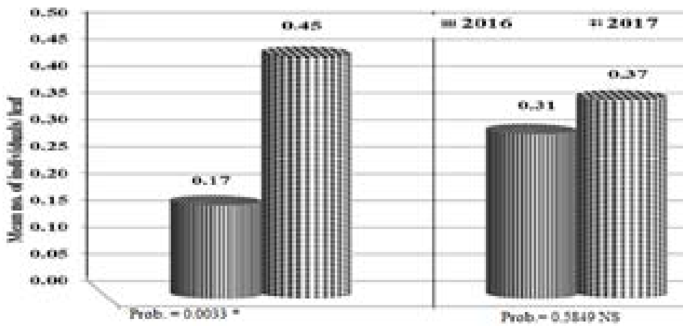


Fig. 2: population density of mirid bug, *N. tenuis* and *C. undecimpunctata* during summer seasons, 2016 and 2017.

Successively similar studies, weekly field observations of *N. tenuis* and its prey *B. tabaci* were recorded by Sridhar *et al.* (2012) on two tomato varieties. Earlier studies were a high interest for the utilization of the predator in biological control programmes which *N. tenius* was reported to prey on *B. tabaci* and several other pests including *Tetranychus urticae*، *Spodoptera litura*, *Ephestia kuehniella* and *Tuta absoluta* (Sancheza and Lacasa, 2008; Perdikis *et al.*, 2009). Despite its importance in biological control, information on incidence of *N. tenuis* was scanty in Egypt. It needs several studies on the risk assessment of this predator on the vegetable plant, because it causes necrotic rings around leaf petiole, growing shoots, flower drop and spots on fruit. So that, it may be need to detect the prey consumptions before the release this predator to avoid the predator risks.

Data in Table (2) illustrated that the correlation coefficient value (r) was showed a positive relation between *N. tenuis* population and two pests, *A. gosspyii* and *B. tabaci* populations. The r values were ranged from 0.26 to 0.81 that mean, the increase of these pests increase the *N. tenuis* density. The explained variance (E.V. %) was 9.79% & 34.44% with *A. gosspyii* individuals and 6.74 & 64.93% with *B. tabaci* nymphs during seasons, 2016 and 2017, respectively (Table 2). However, Sridhar *et al.* (2012) found that *N. tenuis* population had been nonsignificant negative correlation with the incidence of *B. tabaci* populations. Shanchez (2009) was reported inversely interaction between the number of *N. tenuis* and the number of whitefly immatures.

A high significantly of *C. undecimpunctata* was observed during seasons, 2016 and 2017 being 0.17 and 0.45 individuals/ leaf, respectively (probability= 0.0033) (Fig. 2). Statistical analysis conducted that *C. undecimpunctata* was related by a positive relation with *A. gosspyii* populations (r values= 0.85 and 0.44 with E.V. % values = 71.50% and 18.98% during summer seasons, 2016 and 2017, respectively) (Table 2). Similarly, eleven spotted ladybird beetle, *C. undecimpunctata* was an important and successful predator of a number of pests attacking cotton, sunflower, citrus and vegetables crops (Saeed *et al*., 2007). Pereira and Smith (1976) indicated that Coccinellidae was found preying on aphids infesting squash. The predators; mirid bug, *N. tenuis* (Heteroptera: Miridae) and eleven spotted ladybird beetle, *C. undecimpunctata* (Coleoptera: Coccinellidae) were the most abundant predatory species surveyed in squash fields. In conclusion, the occurrence of these predators observed in squash fields were synchronized with major piercing sucking insect pests viz. *B. tabaci* and *A. gossypii*. Thus, indicating the important natural role of biocontrol agents against these pests. Magnifying this role, it may be need to release of *N. tenuis* and *C. undecimpunctata* in squash fields and also other fields that are attacked by the major piercing sucking insect pests when planning IPM strategies against them.

Table 2: Correlation between *B. tabaci* and *A. gosspyii* populations and their damage symptoms and associated predators.

Pest	Stages	Season	Damage symptoms						Associated predators					
			No. of Silvering leaves/ 10 plants			No. of virus / 50 plants			Mirid bug, *Nesidiocoris tenuis*			Ladybird, *Coccinella undecimpunctata*		
			r	Prob.	E.V.%	r	Prob.	E.V.%	r	Prob.	E.V.%	r	Prob.	E.V.%
Aphid, *Aphis gossypii*	Individuals	2016	——			0.48	0.16	22.75	0.31	0.38	9.79	0.85	0.002	71.5
		2017				0.14	0.7	2.01	0.59	0.07	34.44	0.44	0.21	18.98
Whitefly, *Bemisia tabaci*	Nymphs	2016	0.18	0.63	3.06	——			0.26	0.47	6.74	——		
		2017	0.21	0.57	4.26				0.81	0.005	64.93			
	Adults	2016	0.07	0.84	0.56				——					
		2017	-0.08	0.82	0.68									

r= Correlation coefficient E.V.% : Explained variance Prob. = Probability

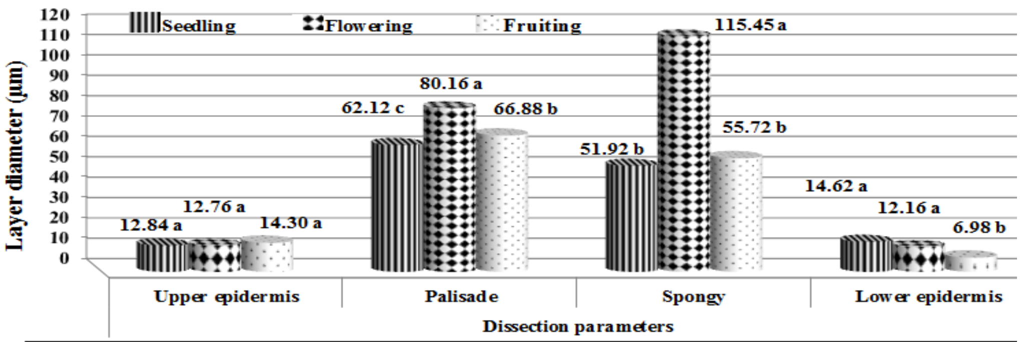
The relationship between the population fluctuation of *B. tabaci* and number of silvering leaves & *A. gosspyii* populations and number of mosaic virus was also studied through the correlation coefficient value (r) and explained variance (E.V. %) on squash plant during summer season, 2016 and 2017 (Table 2). In case of *A. gosspyii*, the relationship was insignificantly positive with number of mosaic virus on squash plant with r value was 0.48 and 0.14 during summer seasons 2016 and 2017, respectively. The explained variances (E.V. %) were

22.75% and 2.01% during two seasons, respectively (Table 2). Also, there was a positive and non-significant between *B. tabaci* (adults & nymphs) and the number of silvering leaves on squash plant except in adult stages during summer season, 2017. Correlation coefficient value (r) was 0.18 & 0.21 and 0.07 & -0.08 during summer seasons, 2016 and 2017, respectively. The explained variance in case of *B. tabaci* individuals ranged from 0.56% to 4.26% (Table 2). Most likely as in the present work, mosaic virus is an aphid- potyvirus, regarded a major pathogen of cucurbits in most regions of the world (Provvident *et al*., 1984).

Effect of some dissection structures of squash plant:

Thickness of leaf is a character that contributes towards resistance and hence effects pest infestation. So, the population densities of whitefly, *B. tabaci* and cotton aphid, *A. gosspyii* individuals infesting squash leaves were related to the dissection characters of squash leaves, such as the thickness of the leaf layers (upper epidermis, palisade، spongy tissues and lower epidermis). This part of study was done to discover the correlation between these layers and the mean population density of two pests at three growth stages during the two studied seasons (Fig. 3).

Data illustrated in Fig. (3) showed the differences between the thickness of the squash leaf layers at three growth stages (seedling, flowering and fruiting). The present results showed a significant differences between three growth stages for dissection structures of squash plant (palisade, spongy and lower epidermis layers), but it was insignificant in case of upper epidermis. Concerning the statistical analysis of the obtained data, the results showed that the relationship between *A. gosspyii* populations and the dissection structures was a positive relation with palisade, spongy and lower epidermis layers, being r value equal 0.85, 0.94 and 0.49 with the explained variance (E.V.%) equal 71.68%, 87.78% and 23.54%, respectively. But, the relation was a negative with upper epidermis layer (r= -0.77 & E.V. %= 58.77%) (Fig.3). An insignificant positive correlation coefficient values were scored between the mean numbers of *B. tabaci* individuals infesting squash plant *C. pepo* in related to thickness of upper epidermis, palisade and spongy layers (being r value = 0.34, 0.79 and 0.62 with E.V.%= 11.34, 62.81 and 42.57%, respectively).



		Upper epidermis	Palisade	Spongy	Lower epidermis
F value		0.72	61.60	980.20	24.53
LSD		3.537	4.123	3.938	2.727
Aphid	r	-0.77	0.85	0.94	0.49
	Prob.	0.44	0.35	0.23	0.68
	E.V.%	58.77	71.68	87.78	23.54
White fly	r	0.34	0.79	0.62	-0.65
	Prob.	0.78	0.42	0.55	0.55
	E.V.%	11.34	62.81	42.57	42.54

Fig. 3: Relationship between dissection structures of squash plant leaves and *B. tabaci* and *A. gosspyii* infestations.

However, a negative relation (r = -0.65 & E.V. % = 42.54%) was recorded for lower epidermis with the population of *B. tabaci* on squash plant.

The obtained results were similar to issue was reported by Abou-Zaid (2013), who studied the population of red spider mite was related to dissection structures of 5 cucumber varieties such as upper epidermis, spongy palisade and lower epidermis layers at two growth stages, seedling and flowering+ fruiting. Hanafy *et al.* (2014) determine the host preference of *C. sativus* to certain pests including, thrips, aphids, whitefly, and its relationship with anatomical characters. They found that the population density of all pests had positive relationship and significant with palisade and spongy layers. On the other hand, this relation was significantly negative with upper and lower epidermis. Among 29 *Cucumis melo* cultivars and lines screened for resistance to *A. gossypii* was conducted by Kitroongruang *et al.* (1991), who studied that leaf anatomy was related with *A. gosspyii* populations. However, the relation between leaf thickness and mid-vein thickness with *A. craccivora* populations was detected by Abd El-Zaher *et al.* (2016). Finally, the dissection structures of squash leaf effect on the population of certain sucking and piercing pests as *B. tabaci* and *A. gosspyii* during summer plantations.

Effect of certain plant enzymes and chlorophyll content.

One of the important aspects of host plant resistance against insects is the disruption of insect's nutrition. The enzymes organized the nutrient uptake by insects through formation of electrophiles including Alpha esterase, Peroxidase and Phenoloxidase. In this part, the correlation between these enzymes and the infestation of the two investigated pests was conducted. Data tabulated in Table (3) indicated that the amount of Alpha esterase was higher in seedling stage (5583.70) than other stages. The lowest amount of Alpha esterase was recorded at fruiting stage (2249.70). While the high amount of Peroxidase was reported at flowering stage (37.70 and 38.10 during summer, 2016 and 2017 with overall mean being 37.90, respectively. Also, the highest level of Phenoloxidase was recorded at flowering stage (9.33). The lowest level of Peroxidase and Phenoloxidase was noticed at the fruiting stage with overall mean being 5.86 and 7.33, respectively, there were significant differences between them (Table 3). Data in Table (4) illustrated that a non-significantly negative relation was observed between seasonal fluctuations of *A. gosspyii* and *B. tabaci* individuals with the level of Alpha esterase in squash plant. The correlation coefficient (r) of Alpha esterase showed their highest value (-0.99) with *B. tabaci* population and their lowest ones (-0.27) with *A. gosspyii* population.

Table 3: levels of certain plant enzymes at three growth stages of squash plant during two successive seasons, 2016 and 2017.

stages	Plant enzymes								
	Alpha esterase ($\mu g\alpha$-naphthol/min/gdw)			Peroxidase (ΔO.D.$_{430}$/min/gdw)			Phenoloxidase (ΔO.D.units/min/gdw)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Seedling	5586.67 ^{a}	5580.67 ^{a}	5583.70 ^{a}	10.00 ^{b}	10.40 ^{b}	10.20 ^{b}	8.53 ^{a}	8.93 ^{a}	8.73 ^{a}
Flowering	2252.67 ^{b}	2246.67 ^{b}	2441.70 ^{b}	37.70 ^{a}	38.10 ^{a}	37.90 ^{a}	9.13 ^{a}	9.53 ^{a}	9.33 ^{a}
Fruiting	2444.67 ^{b}	2438.67 ^{b}	2249.70 ^{b}	5.66 ^{c}	6.06 ^{c}	5.86 ^{c}	7.13 ^{b}	7.53 ^{b}	7.33 ^{b}
F value	152.89	153.26	153.08	282.68	274.18	278.58	11.42	16.34	13.59
L.S.D.	523.89	523.24	523.55	3.5774	3.6324	3.6037	1.0509	0.8785	0.9634

Values signed by the same letter in the same column are non-significantly different at alpha=0.05 level.

Table 4: Correlation between certain plant enzymes and *B. tabaci* and *A. gosspyii* infestations during two successive seasons, 2016 and 2017.

Pest	Season	Plant Enzymes								
		Alpha esterase			Peroxidase			Phenoloxidase		
		r	Prob.	E.V.%	r	Prob.	E.V.%	r	Prob.	E.V.%
Aphid, *A. gossypii*	2016	-0.44	0.71	19.52	0.99	0.005	99.9	0.81	0.40	64.98
	2017	0.21	0.86	4.55	0.79	0.42	61.94	0.99	0.03	99.84
	Mean	-0.27	0.83	7.17	0.98	0.11	96.84	0.90	0.28	81.33
Whitefly, *B. tabaci*	2016	-0.97	0.17	93.40	0.65	0.55	42.44	0.08	0.95	0.71
	2017	-0.99	0.05	99.44	0.37	0.76	13.38	0.25	0.84	6.16
	Mean	-0.99	0.05	99.32	0.51	0.66	25.76	0.09	0.94	0.86

r= Correlation coefficient E.V.% : Explained variance Prob. = Probability

The explained variance (E.V.%) was 99.32% and 7.17% with *B. tabaci* and *A. gosspyii* populations, respectively, that mean the increase of two pests decrease this enzyme (Table, 4). Otherwise, a non-significantly positive relation was noticed between level of both Peroxidase and Phenoloxidase enzymes and Aphid and whitefly densities per squash leaves (0.98 & 0.90 with *A. gosspyii* density and 0.51 & 0.09 with *B. tabaci* density, respectively) (Table 4).

The E.V. % was 96.84% and 81.33% in case of *A. gosspyii* with Peroxidase and Phenoloxidase enzymes in squash leaves, respectively. In case of whitefly, the E.V. % was 25.76% and 0.86% with Peroxidase and Phenoloxidase enzymes, respectively (Table, 4). Plant structures are the first line of defense against herbivore, and play an important role in host plant resistance to insects. The nutrition disruption of insects is the important aspects of host plant resistance against insects. The enzymes impaired the nutrient uptake throughout by Peroxidases and Phenoloxidases. Induction of anti-oxidative enzymes in plants induced host plant resistance (Dunse *et al*., 2010). These present results provide support for those previously reported by Zhou Fu Cai (2013), who found that Peroxidase isozyme, Polyhenoloxidase, Phenyalanine ammonialyase and cholinesterase activities raised faster with peak higher of aphid in resistant than susceptible cucumber varieties. Liang *et al.* (2016) conducted that the Malondialdehyde content, Peroxidase isozyme and Polyhenoloxidase activities were likely to be involved in aphid-resistance in cucumber plants. The results suggested that the enhanced activities of the enzymes may be contributing to bio-protection of squash plants against *A. gosspyii* and *B. tabaci* infestations.

The results in Table (5) revealed that a significantly variances between three investigated plant enzymes, Alpha esterase, Peroxidase and Phenoloxidase in green and silvering squash leaves during two successive summer seasons, 2016 and 2017 being 2327.7 & 1195.7 of Alpha esterase, 5.53 & 3.53 of Peroxidase and 7.53 & 9.1 of Phenoloxidase in green and silvering squash leaves, respectively (Table 5). Furthermore, a non-significantly variance was recorded between the levels of chlorophyll in green and silvering squash leaves during summer seasons, 2016 and 2017 (Mean 1.29 and 1.65, respectively).

Through the view of those results, the chlorophyll cotent was observed higher in silvering leaf than green leaf. These results indicated that the silvering squash leaves were not due to decrease the level of chlorophyll in squash plant. This phenomenon of silver squash leaf disorder may be due to presence of air space between the upper epidermis and palisade layers resulting in altered reflection of light. It is more likely that results were reported by Burger *et al.* (1988), Jimenez *et al.* (1995). Once a leaf is turned into silver colour, it remains silver. In addition, the reduced chlorophyll levels could be responsible for a reduction in photosynthesis (Jimenez *et al*., 1995).

Table 5: Comparison between levels of certain plant enzymes and chlorophyll content in green and silvering squash leaves.

Leaf	Plant enzymes									Chlorophyll content (mg/gdw)		
	Alpha esterase (μgα-naphthol/min/gdw)			Peroxidase (ΔO.D.$_{430}$/min/gdw)			Phenoloxidase (ΔO.D.units/min/gdw)					
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Green	2330.67	2324.67	2327.7	5.33	5.73	5.53	7.33	7.73	7.53	1.3	1.28	1.29
Silver	1198.67	1192.67	1195.7	3.33	3.73	3.53	8.9	9.3	9.1	1.65	1.64	1.65
Prob.	0.0001*	0.0001*	0.0001*	0.004*	0.01*	0.005*	0.01*	0.01*	0.02*	0.01*	0.27 NS	0.11 NS

Analysis according to t-test procedure by SAS program (2003)

Accordingly, screening of aphid and whitefly densities per leaves may be helpful to obtain a suitable time for controlling these pests in squash plants. Also, we may conclude that the natural enemies and plant enzymes may be helpful to obtain a good IPM elements against arthropod pest development on squash plant during summer plantations.

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ARABIC SUMMERY

علاقة الإصابة بالذبابة البيضاء *Bemisia tabaci* و المنّ *Aphis gosspyii* ببعض الأمراض النباتية ، نشاط الإنزيمات النباتية، الصفات التشريحية و الأعداء الحيوية على نبات الكوسة

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تعتبر الذبابة البيضاء والمنّ من أهم الآفات الحشرية بمحصول الكوسة وتسبب أضراراً عديدة منها حدوث أمراض فيروسية وتغيرات فسيولوجية للنبات مثل الورقة الفضية وفيروس الموزيك. وقد تناولت هذة الدراسة دراسة علاقة الإصابة بكل من الذبابة البيضاء *Bemisia tabaci* و المنّ *Aphis gosspyii* وبعض الأمراض النباتية، نشاط الإنزيمات النباتية، الصفات التشريحية و الأعداء الحيوية على نبات الكوسة. وتم إجراء تجربة حقلية بقرية منشية سقارة، الجيزة، مصر خلال الموسم الصيفى ٢٠١٦ و٢٠١٧ .

وقد أظهرت النتائج وجود إرتباط بين نشاط بعض الإنزيمات مثل ألفا إستريز، بيروكسيديز و فينولوأكسيديز والمحتوى النباتى من الكلورفيل والإصابة بكل من الذبابة البيضاء *Bemisia tabaci* و المنّ *Aphis gosspyii*، وأيضا تم دراسة إرتباط بعض الأعداء الحيوية المصاحبة مثل *Nesidiocoris tenuis* و *Coccinella undecimpunctata* وكل من الذبابة البيضاء والمنّ. تم تقدير درجات نمو الورقة الفضية على نبات الكوسة. وقد بينت النتائج أن حدوث الورقة الفضية تشبه السمية النباتية للورقة. وقد أوضحت الدراسة أيضا أن فى حالة الإصابة الخفيفة يكون اللون الفضى منتشراً بين العرق الرئيسى والعروق الثانوية فى الورقة على السطح العلوى، أما فى حالة الإصابة الشديدة فيكون السطح العلوى للورقة فضى اللون بينما يكون السطح السفلى للورقة طبيعياً.